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Physiological Responses of *Salvinia minima* to Different Phosphorus and Nitrogen Concentrations

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Abstract.—This study was designed to evaluate the effectiveness of salvinia (*Salvinia minima*) in accumulating nitrogen and phosphorus under different imitating eutrophic environments. Nitrogen concentrations of 1.0 (control), 10.0, and 100 mg/l and phosphorus concentrations of 0.1 (control), 1.0, and 10.0 mg/l were used in this study. Plants were grown under laboratory conditions at 25 ± 2°C with a light intensity of 120 μmol/m^2/s, and a 14 hr photoperiod. Salvinia's growth, expressed as frond production and plant fresh weight doubling time, was significantly increased with increasing nitrogen concentration from 1.0 mg/l to 100.0 mg/l in the growth media. The increase in growth rate was independent from the variation in phosphorus concentrations. However, the highest growth rate was obtained for days 1 through 7 when the levels of both nitrogen and phosphorus were elevated 100 fold (100 mg/l N and 10.0 mg/l P) from that of control treatments. This treatment also resulted in the highest photosynthetic rate, chlorophyll *a* and *b* content, carotenoids and anthocyanins concentrations. Nitrogen and phosphorus concentration did not influence soluble sugar (SS) accumulation. Starch and total-nonstructural carbohydrate (TNC) accumulation was significantly lower in treatments receiving elevated levels of nitrogen or phosphorus when compared to the control. The highest uptake of nitrogen and phosphorus into plant tissues resulted when both nutrients were elevated 100 fold (100 mg/l N and 10.0 mg/l P) and were higher at day 14.

Key Words.—*Salvinia minima*, CO_2 assimilation, photosynthetic pigments, nitrogen, phosphorous, eutrophication

According to the USEPA Clean Water Act of 1998, eutrophication has become a major water pollution problem worldwide (Litke, 1999; USGS, 1999). Eutrophication can be hastened dramatically by human interactions with the environment, such as excessive fertilizer runoff, animal feedlot operations, sewage, and industrial waste (Krohne, 1998). In eutrophic lakes and streams, phosphorus and nitrogen are generally considered to be the limiting nutrients and excessive levels greatly accelerate eutrophication (Likens, 1972; Schindler, 1975; USGS, 1999). The combined elevated concentrations of nitrogen and phosphorus was associated with a dramatic increase in eutrophication rate more than if only one these nutrients were present alone (Likens, 1972). Phosphorus is generally found in water in the form of phosphates and orthophosphate (dissolved phosphate), $H_2PO_4^-$ and HPO_4^{2-} (Manahan, 1994). The USEPA has established a recommended limit of 0.05 mg/l for total

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phosphorus in streams that enter lakes and 0.1 mg/l for total phosphorus in flowing waters (USEPA, 1986).

Nitrogen is found in waterways primarily in the form of nitrates (NO^{-3}) and, less commonly, ammonia (NH^{+4}) (USGS, 1999). Like phosphorus, it enters the water through agricultural, urban, and industrial runoff. To prevent nuisance plant growth, an ideal level of total nitrogen (nitrates, nitrites, and ammonia) of 1.0 mg/l should not be exceeded (USGS, 1999). It was estimated that 61% of sampled streams were enriched with total nitrogen and nitrate, and 23% were enriched with ammonia.

One possible solution for eutrophication is the use of aquatic plants as bioaccumulators of nutrients into organic forms, thus reducing the levels of the nutrients in question in the aquatic environment. A variety of aquatic plants have been investigated for their ability to reduce heavy metal toxicity and reduce nutrient concentration in the aquatic environment (Reddy and DeBusk, 1985; Gardner and Al-Hamdani, 1997; Chau, 1998). Among the major considerations when selecting an aquatic plant as a bioaccumulator of nutrients are acceptable tolerance levels to the elements in question and a relatively high growth rate. Salvinia (*Salvinia minima* Baker) is a small, free-floating freshwater macrophyte widely distributed in tropical and temperate regions of the world (Nauman, 1993). Under favorable environmental conditions, salvinia spreads by vegetative reproduction and is capable of colonizing large areas of water in a short period of time (Gaudet, 1973). The rapid growth of salvinia has been considered a problem in the field, and for this reason, salvinia is classified as a nuisance weed. However, with the increased interest in the use of aquatic plants for the removal of excess nutrients from wastewater, the aggressive growth of salvinia can be considered a positive characteristic. This quality satisfies a major requirement in plant selection for phytoremediation or nutrient removal (Salt *et al.*, 1995). Salvinia also possesses additional positive characteristics. Under controlled conditions, salvinia acts as a strong buffering agent, correcting the pH of its media from 3.9 to 6.8 within 24 hours (Gardner and Al-Hamdani, 1997). It can also withstand relatively high concentrations of Al (20 mg l^{-1}) (Gardner and Al-Hamdani, 1997). In addition, salvinia was found to survive under relatively high concentrations of Cr (1 mg l^{-1}). Salvinia experienced significant Cr uptake without adverse effects on its growth (Nichols *et al.*, 2000). Therefore, the objectives of this study were to evaluate the growth of salvinia and its effectiveness in accumulating nitrogen and phosphorus into its tissues under different eutrophic environments. Nitrogen concentrations of 1.0, 10.0 and 100.0 mg/l, and phosphorus concentrations of 0.1, 1.0, and 10.0 mg/l were used in this study. These concentrations were selected to represent the wide range of possibilities found in nature as well as the levels commonly found in sewage treatment plants (Litke, 1999). The selection of these concentrations was an attempt to evaluate the possibility of using *Salvinia* as a biological agent to remediate nitrogen and phosphorus in a eutrophic environment. The influence of these concentrations of nitrogen and phosphorus on salvinia growth, photosynthetic rate, photosynthetic pigments, anthocyanin, and

TABLE 1. Experimental treatment levels of nitrogen and phosphorus in the growth media. Treatment levels selected to mimic levels of nitrogen and phosphorus found in nature (T1 and T2) as well as levels found in sewage effluent (T5). The effect of elevating each nutrient separately was also considered (T3 and T4).

Treatment	Nitrogen (mg l^{-1})	Phosphorus (mg l^{-1})	N:P*
T1	1.0	0.1	10:1
T2	10.0	1.0	10:1
T3	100.0	0.1	1000:1
T4	1.0	10.0	1:10
T5	100.0	10.0	10:1

*Represents ratios of nitrogen and phosphorus in the growth media, respectively, as compared to control, first level of treatment (10:1).

spectral properties were evaluated. The evaluation of these selected physiological responses was used to assess the impact of the elevated concentrations of the nitrogen and phosphorus on salvinia.

MATERIALS AND METHODS

To accomplish the objective of this study, two separate experimental settings were designed:

Experiment 1.—Salvinia plants with a total of 15 fronds were placed in 225 ml Erlenmeyer flasks containing 125 ml of modified Hoagland's Solution (Hoagland and Arnon, 1938). Nitrogen was provided in the form of calcium nitrate, $Ca(NO_3)_2$, potassium nitrate, $K(NO_3)$, and magnesium nitrate, $Mg(NO_3)_2$. Phosphorus was provided in the form of potassium phosphate, $KH_2PO_4^-$. Five treatments were selected to mimic trophic states found in nature as well as levels found in water treatment sites (Table 1). The control treatment contained 1.0 mg/l N and 0.1 mg/l P which represents desired regulatory levels found in nature (USGS, 1999). The nitrogen and phosphorus concentrations were increased ten fold (10.0 and 1.0 mg/l, respectively) to represent the elevated concentrations found in nature that result in eutrophication. Levels consistent with those in sewage treatment plants were accomplished by increasing N and P 100 fold (100 mg/l and 10.0 mg/l, respectively). In addition, two selected treatments were included in this study in order to manipulate either nitrogen or phosphorus in the growth media: one variation included high levels of nitrogen (100 mg/l) and ideal levels of phosphorus (0.1 mg/l), while the other included high levels of phosphorus (10.0 mg/l) and ideal levels of nitrogen (1.0 mg/l).

Six samples per treatment (each with a 15 fronds), 30 samples total, were randomly placed in the growth chamber under 120 μmol m^{-2} s^{-1} photon flux density, a 14 hr photoperiod, and a temperature of 25 ± 2°C. The initial plant fresh weight and leaf number was rec orded and plants were allowed to grow for 14 days. The plant fresh weight and leaf number of each sample was recorded on days 7 and 14 of the experiment. At the conclusion of the

experiment, plant growth, photosynthetic rate, chlorophyll *a* and *b*, carotenoid, anthocyanin, and carbohydrates were evaluated.

Growth was determined using doubling time (DT) in terms of plant fresh weight and leaf number. The doubling time values were calculated using the following formula: $DT = t \log 2/\log (w_t\ w_o^{-1})$ (Moretti and Gigliano, 1988). Where DT was the doubling time (in days), t was the experiment duration (in days), w_t was the final weight (g) or leaf number, and w_o was the original weight (g) or leaf number.

Carbon dioxide assimilation and internal CO_2 concentration of six randomly selected plants from each replicate of each treatment were measured four hours after the onset of the light period at day fourteen of the treatment application. The selected frond of each sample was enclosed in a flow-through plexiglass assimilation chamber (4.5 × 11.8 × 7.3 cm) of a Li-Cor 6200 photosynthesis system (Lincoln, NE, USA), as described by McDermitt *et al.* (1989). Standard measurement conditions were 150 μmol m^{-2} s^{-1} photon flux density, 45–50% RH, and 25°C.

One gram of fresh weight from each sample was placed in a 10 ml vial containing 5 ml of DMF (N, N-Dimethylformamamide) solution and incubated in the dark for 36 hours at 4°C. Chlorophyll *a* and *b* concentrations were determined spectrophotometrically, at A_{647} and $A_{664.5}$, using the formula of Inskeep and Bloom (1985). Carotenoid concentrations were determined from the same DMF extract at A_{470}, and the concentrations were calculated using the formula of Doong *et al.* (1993).

Plant samples of 1.0 g fresh weight were homogenized, using a mortar and pestle, in 5 ml methanol containing 1% HCl. The samples were centrifuged for 5 min. at 3000 rpm. The absorbance of the supernate was determined spectrophotometrically at A_{530} (peak absorbance of anthocyanin) and at A_{637} (peak absorbance of degraded products of chlorophyll in acidic methanol). Anthocyanin concentrations were calculated using the formula developed by Doong *et al.* (1993) and Mancinelli (1990).

Carbohydrate analysis of the plant samples was conducted following a procedure slightly modified from Chatterton *et al.* (1987). Samples were ground into a fine powder and a 100–500 mg portion was placed in a sealed vial and used for the determination of soluble sugars (SS), starch, and total nonstructural carbohydrates (TNC).

Experiment 2.—A separate experiment was carried out to evaluate the nitrogen and phosphorus uptake. This experiment was conducted similar to experiment 1, with the exception that salvinia plants with a total of 733 fronds were placed in 10-gallon containers with 11 liters of 10% Hoagland's solution with identical nitrogen and phosphorus concentrations as were in first experiment. This was to insure adequate supply of plant samples needed for chemical analysis.

Dried plant tissues of each sample from each treatment were weighed and placed in 100 ml Pyrex beakers. Each sample was digested in nitric acid (15.8 N), hydrogen peroxide (30%) and hydrochloric acid (15.8 N) following the procedure outlined by Cabrera-Vique *et al.*, (1997). After digestion, the

TABLE 2. Growth of salvinia as influenced by various concentrations of nitrogen and phosphorus in the growth media. Plant growth expressed as doubling time based on frond number and plant fresh weight.

Treatment (N:P)	Doubling Time (days)			
	Frond Number		Plant Fresh Weight (mg)	
	length of exposure (Days)			
	1–7	1–14	1–7	1–14
1.0/0.1	6.2a	9.4a	4.2a	6.4a
10.0/1.0 (10:10)*	5.4b	7.1b	3.8ab	5.0b
100.0/0.1 (100:0)	4.5c	5.8c	3.8ab	4.8b
1.0/10.0 (0:100)	5.9b	9.1a	4.1a	6.2a
100.0/10.0 (100:100)	4.3c	5.9c	3.3b	4.8b

Means followed by the same lowercase letter in each column are not significantly different based on the LSD test (P = 0.05). Each value is the mean of six replications.
*Numbers in parentheses represent ratio of nitrogen and phosphorus increase (fold) as compared to control (1.0/0.1).

samples were diluted to 100 ml with distilled water. Phosphorus concentrations were determined using a Perkin-Elmer 1100 B Graphite Furnace Atomic Absorption spectrophotometer.

Total nitrogen analysis was carried out using a modified Dumas method as outlined by Saint-Denis and Goupy (2004).

Experiments were each repeated twice and analyzed statistically as a randomized complete design (Steel and Torrie, 1980). This design ensured that observed differences in plant performances were due to treatments rather than variations among blocks (replicate series was conducted at different times). Treatments that showed significant F values (P = 0.05) based on ANOVA analysis were separated based on the least significant difference (LSD) test (Steel and Torrie, 1980).

RESULTS

In comparison to the control, increasing the nitrogen and phosphorus concentration 10 fold resulted in a reduction in time required for the frond number to double (days 1–7) from 6.2 to 5.4 days (Table 2). When phosphorus alone was increased 100 fold, frond doubling time was 0.3 days less than the control. Independent elevation of the nitrogen concentration to 100.0 mg/l resulted in 27.4% reduction in total days required for doubling the population. However, the increase in both nitrogen and phosphorus to 100 fold resulted in a growth rate equal to the elevation of nitrogen alone 100 fold. Elevating phosphorus independently 100 fold resulted in growth rates equal to the growth rates obtained by elevating both nitrogen and phosphorus 10 fold. When comparing the growth rates during days 1–7 with the growth rates during days 7–14, similar growth rates between treatments resulted with the exception being a decrease in growth when phosphorus concentration alone

TABLE 3. Chlorophyll *a* (chl *a*) and chlorophyll *b* (chl *b*) concentration in salvinia growing for 14 days at various nitrogen and phosphorus concentrations.

N/P (mg l^{-1})	chl *a*	chl *b*	chl *a*/chl *b*
	mg/g Fresh weight		
1.0/0.1	3.68a	2.64a	1.63a
10.0/1.0 (10:10)*	6.04b	4.21b	1.44a
100.0/0.1 (100:0)	8.61c	5.58c	1.54a
1.0/10.0 (0:100)	3.80a	2.84a	1.33a
100.0/10.0 (100:100)	19.66d	9.94d	1.98b

Means followed by the same lowercase letter in each column are not significantly different based on the LSD test (P = 0.05). Each value is the mean of six replications.
*Numbers in parentheses represent ratio of nitrogen and phosphorus increase (fold) as compared to control (1.0/0.1).

was elevated 100 fold. Calculating salvinia growth using the fresh weight value showed similar growth among the treatments receiving the various concentrations of nitrogen and phosphorus during the first week of the experiment (Table 2), the exception being those plants grown at 100 fold concentrations of nitrogen and phosphorus, which exhibited significantly higher growth rates when compared to the control. However, calculating the growth for the experiment duration, salvinia's doubling time was significantly reduced by increasing the nitrogen concentration from 1.0 to 100.0 mg/l in the growth media. The increase in salvinia growth rate was independent from the variation in phosphorus concentration, and was similarly influenced by the elevation of nitrogen concentrations from 1.0 mg/l.

Introducing salvinia to nitrogen concentrations higher than 1.0 mg/l significantly influenced the increase in chlorophyll *a* and *b* concentrations (Table 3). Chlorophyll *a* increased 1.64 and 2.34 fold in comparison to the control when nitrogen was elevated to 10.0 and 100.0 mg/l, respectively. The independent increase in phosphorus concentration in the growth media was shown to have a similar impact on chlorophyll *a* and *b* as compared to the control. However, the highest significant increase in both chlorophylls was obtained when nitrogen and phosphorus were both elevated 100 fold. The impact of the variation of nitrogen and phosphorus concentration was equal on chlorophyll *a* and *b*, as was shown by similar values for chlorophyll *a/b* ratios (Table 3). However, the 100 fold combined elevation of both nutrients had a more significant impact on chlorophyll *a* than *b*, resulting in a higher chlorophyll *a/b* ratio.

The concentration of carotenoid increased with increasing nutrient concentrations, although the increase was not statistically significant until the 100 fold level of both nitrogen and phosphorus was reached (Table 4). Anthocyanin concentrations were significantly increased with the 100 fold increase of nitrogen alone, as well as when nitrogen was elevated in combination with 100 fold concentrations of phosphorus (Table 4).

TABLE 4. Carotenoids and anthocyanins concentrations in salvinia growing for14 days at various nitrogen and phosphorus concentrations.

N/P (mg l^{-1})	Carotenoids (µg/g FW)	Anthocyanins (µg/g FW)
1.0/0.1	162.33a	0.56a
10.0/1.0 (10:10)*	562.41a	1.28a
100.0/0.1 (100:0)	1568.98a	4.50b
1.0/10.0 (0:100)	183.35a	0.34a
100.0/10.0 (100:1)	10939.37b	7.55c

Means followed by the same lowercase letter in each column are not significantly different based on the LSD test (P = 0.05). Each value is the mean of six replications.
*Numbers in parentheses represent ratio of nitrogen and phosphorus increase (fold) as compared to control (first level of treatment).

The variation in nitrogen and phosphorus concentration did not influence soluble sugar accumulation in salvinia (Table 5). Treatments receiving elevated levels of nitrogen or phosphorus were significantly lower in starch accumulation in comparison to the control. In general, total nonstructural carbohydrate levels followed a pattern similar to starch accumulation.

After 7 days of salvinia growth at different nitrogen and phosphorus concentrations, the photosynthetic rate significantly increased in those treatments receiving a 100 fold increase in nitrogen concentrations (Table 6). The increase in photosynthetic rate was independent from the variation in phosphorus concentration. Similar results were obtained at day 14 of the experiment, with the exception that increasing the nitrogen 10 fold and higher significantly enhanced the photosynthetic rate. Reduction in photosynthetic rate was obtained at day 14 when compared to day 7 in those treatments receiving 1.0 mg/l nitrogen and those treatments receiving 100 fold concentrations of both nitrogen and phosphorus. Variation in photosynthetic rate among the treatments was not impacted by availability of carbon dioxide, as indicated by the insignificant difference in internal carbon dioxide concentrations in all treatments at days 7 and 14 (Table 6).

TABLE 5. Soluble sugars (SS), starch, and total non-structural carbohydrate (TNC) accumulation in salvinia growing for14 days at various nitrogen and phosphorus concentrations.

	SS	Starch	TNC
N/P (mg l^{-1})		mg g^{-1} dry weight	
1.0/0.1	25.45a	174.98a	303.24a
10.0/1.0 (10:10)*	43.00a	118.31b	243.11b
100.0/0.1 (100:0)	21.05a	110.03b	196.32b
1.0/10.0 (0:100)	28.88a	129.16b	248.10bc
100.0/10.0 (100:100)	19.90a	106.28b	186.18b

Means followed by the same lowercase letter in each column are not significantly different based on the LSD test (P = 0.05). Each value is the mean of six replications.
*Numbers in parentheses represent ratio of nitrogen and phosphorus increase (fold) as compared to control (1.0/0.1).

TABLE 6. Carbon dioxide assimilation and internal CO_2 in salvinia growing for14 days at various nitrogen and phosphorus concentrations.

N/P (mg l^{-1})	Photosynthesis (μmol m^{-2} s^{-1})		Internal CO_2* (μl l^{-1})	
	day			
	7	14	7	14
1.0/0.1	2.75aA	2.00aB	356.83	361.28
10.0/1.0 (10:10)**	3.22aA	3.19bA	358.18	352.30
100/0.1 (100:0)	5.72bA	5.42cA	351.95	348.83
1.0/10.0 (0:100)	2.84aA	1.92aB	364.45	356.55
100/10.0 (100:100)	6.79cA	5.98cB	353.95	353.50

Means followed by the same lowercase letter in each column are not significantly different based on the LSD test (P = 0.05). Upper case letters denote differences between days within treatments.
*Internal carbon dioxide concentration was not significantly different between the treatments.
**Numbers in parentheses represent ratio of nitrogen and phosphorus increase (fold) as compared to control (1.0/0.1).

Salvinia uptake of nitrogen significantly increased with the increase of nitrogen in the growth media (Table 7). The highest significant nitrogen uptake was obtained in treatments receiving 100 fold increased concentrations of both nitrogen and phosphorus. The same results were obtained for day 7 and day 14, with the exception that nitrogen accumulation in salvinia's tissue was significantly higher at day 14. Although salvinia's uptake of phosphorus did not consistently increase with increasing phosphorus concentration, phosphorus levels were statistically significantly higher at day 14 in all treatments when compared to day 7. The presence of elevated concentrations of either phosphorus or nitrogen seemed to significantly influence the other nutrients' uptake. Treatments receiving low nitrogen, 1.0 mg/l, in conjunction with a 100% increase in phosphorus resulted in an 11% increase in nitrogen uptake after 7 days. Those treatments receiving low phosphorus, 0.1 mg/l, in

TABLE 7. Nitrogen and phosphorus content of salvinia at day 7 and 14 grown in various concentrations of nitrogen and phosphorus.

N/P (mg l^{-1})	Nitrogen (% dw)		Phosphorus (mg kg^{-1})	
	Day			
	7	14	7	14
1.0/0.1	1.55aA	2.31aB	0.64aA	0.89aB
10.0/1.0 (10:10)*	1.52aA	2.74bB	0.49bA	0.76bB
100.0/0.1 (100:0)	3.28cA	4.43cB	0.32cA	0.77bB
1.0/10.0(0:100)	1.72dA	1.78dB	0.54dA	0.68cB
100.0/10.0 (100:100)	4.26eA	4.96eB	0.74eA	1.03dB

Means followed by the same lowercase letter in each column are not significantly different based on the LSD test (P = 0.05). Upper case letters denote differences between days within treatments. Each value is the mean of six replications.
*Numbers in parentheses represent ratio of nitrogen and phosphorus increase (fold) as compared to control (1.0/0.1).

conjunction with a 100 fold increase in nitrogen resulted in an 18% increase in phosphorus uptake.

Discussion

Salvinia not only tolerated excess levels of nutrients, but also thrived in this environment (Table 2). This observed increase in growth in the treatment receiving 100 fold nitrogen and phosphorus is consistent with previous research concerning salvinia. Gaudet (1973) similarly concluded that elevated concentrations of nitrogen significantly increase salvinia growth. Salvinia was suggested as viable candidate for treating high-strength organic wastewater with relatively high concentration of ammonium-nitrogen (Olguin *et al.*, 2007). The ability of salvinia to grow in high levels of nutrients, specifically nitrogen and phosphorus, makes it an ideal candidate for growth in a eutrophic environment. Reddy and DeBusk (1985) reported that the growth rate *Salvinia rotundifolia* Wiild. was significantly higher than *Azolla caroliniana* Willd. (azolla), *Spirodela polyrhiza* L. (giant duckweed), and *Lemna minor* L. (common duckweed) under high levels of nutrients. *Salvinia molesta* D.S. Mitch (giant salvinia) has also been shown to grow rapidly in growth media when nitrogen and phosphorus are elevated to 20 mg/l and 2 mg/l, respectively (Toerien *et al.*, 1983).

Salvinia grown in low levels of nutrients (1.0 and 10.0 mg/l nitrogen, 0.1 and 1.0 mg/l phosphorus) exhibited poor growth, and resulted in plants with small, chlorotic leaves. Similar findings were reported for *S. molesta* (Oliver, 1993). In the present experiment, low nutrient levels were considered the control and indicated a desired environment where excess noxious weeds were minimal or absent. The present research concludes that salvinia was not as aggressive in increasing its population under these low nutrient conditions. In addition to growth, all parameters associated directly or indirectly with growth, including levels of chlorophyll *a* and *b*, carotenoid, anthocyanin, carbohydrate accumulation, and photosynthetic rate, were enhanced by excessive nutrient levels in the growth media (Tables 3, 4, 5, 6).

The increase in chlorophyll *a* and *b* concentration was directly correlated to the increase in nitrogen level in the growth media and independent from phosphorus increase (Table 3). However, the highest chlorophyll concentration was obtained when both nitrogen and phosphorus were elevated 100 fold in comparison to control. Nitrogen was added to the growth media in three forms, including $Mg(NO_3)_2$. Because the center of chlorophyll's pyrrole ring consists of Mg^{2+} surrounded by four nitrogen ions, it is postulated that the increase in chlorophyll concentration may have resulted because of the corresponding increase in magnesium provided with the increase in nitrogen in the growth media. It is unknown at this time whether the increase in nitrogen without the corresponding increase in magnesium would produce the same results.

Carotenoids and anthocyanin were significantly elevated in the treatment receiving 100 fold nitrogen and phosphorus. Anthocyanin is a water-soluble

pigment that is found in the vacuole of the cell and functions to protect the plant from damaging ultraviolet rays (Taiz and Zieger, 1998). Carotenoid is an accessory photosynthetic pigment that absorbs light optimally in the 460–550 nm in the visible light spectrum. Its role is to transfer energy to chlorophyll *a* in the light reactions of photosynthesis. It also functions to protect the plant from photooxidation (Hopkins, 1999).

The increase in photosynthetic pigments was directly associated with an increase in photosynthetic rate and with plant growth. Lema *et al.* (2000) reported that chlorophyll concentration in common beans (*Phaseolus vulgaris* L.) was significantly reduced when nitrogen alone was deficient, whereas carotenoid concentration was reduced under both nitrogen and phosphorus limitations. It was reported that the increase in chlorophyll and carotenoid concentration corresponded with an increased rate of photosynthesis in salvinia (Nichols *et al.*, 2000). An increase in photosynthetic rate was also attributed to the influence of nitrogen on enhancing Rubisco content (Quick *et al.*, 1991; Harmens *et al.*, 2000). Elevated photosynthetic rate and plant growth with increased nitrogen concentration was also reported in sunflower, *Helianthus debilis* Nutt. (Pankovic *et al.*, 2000), and in wheat, *Triticum aestivum* L.(Farage *et al.*, 1998).

Photosynthesis and plant growth is directly linked with carbohydrate accumulation into plant tissues. In the current experiment, those treatments receiving 1.0/0.1 mg/l nitrogen and phosphorus, respectively, resulted in a higher accumulation of starch and nonstructural carbohydrate than other treatments (Table 5). In general, the lowest starch and nonstructural carbohydrate accumulation was obtained in treatments receiving elevated levels of nitrogen and phosphorus (100.0/10.0 mg/l). This result coincided with the highest growth rate and photosynthetic rate. This reduction in carbohydrate accumulation should not be interpreted as a decrease in carbohydrate synthesis but as a possible increase in carbohydrate utilization through growth and energy production in supporting cell activities. In comparison, other studies have shown that starch and non-structural carbohydrate accumulation increases in salvinia under stresses including Al (Gardner and Al-Hamdani, 1997) and Cr (VI) (Nichols *et al.*, 2000).

The presence of the elevated concentration of either phosphorus or nitrogen in the growth media seemed to significantly affect nutrient uptake (Table 7). Increasing the nitrogen concentration in the media had a positive influence on plant uptake while the positive effects of increasing phosphorus concentration during days 1–7 was not seen until the 100 fold increased concentrations of both nutrients. These differences in nitrogen and phosphorus uptake after the 14 days of growth could be attributed to the reduction in nitrogen availability resulting from more aggressive growth and faster depletion of nitrogen concentration in the growth media. Phosphorus tissue concentration, growth rate, and photosynthesis were negatively affected in those treatments receiving an increase in phosphorus in the growth media without the corresponding increase in nitrogen (Tables 2, 6, 7). This may be due to the correlation between tissue levels of phosphorus and photosynthetic efficiency. Loustau *et*

al. (1999) demonstrated that needle levels of phosphorus were associated with improved efficiency of carboxylation by Rubisco and improved photochemical efficiency of PSII in pine seedlings of *Pinus pinaster* Ait.

In conclusion, this study demonstrated the ability of salvinia to exhibit optimal growth under elevated concentrations of nitrogen and phosphorus similar to that possibly found under eutrophic conditions. Nitrogen rather than phosphorus was more influential in increasing plant growth and photosynthesis at concentrations and ratios of N:P used in this study. In addition, salvinia possess the ability to incorporate nitrogen and phosphorus into its tissues under these conditions which might be considered as a potential biological agent in remediating nitrogen and phosphorus in eutrophic environments.

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